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(11) EP 0 429 438 B1

(12)

## EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention  
of the grant of the patent:  
28.08.1996 Bulletin 1996/35

(51) Int. Cl. 6: A61L 27/00, C08H 1/06

(21) Application number: 91200300.1

(22) Date of filing: 03.07.1985

### (54) Bone repair using collagen

Kollagenknochenersatzmaterial

Matériau à base de collagène pour la réparation des os

(84) Designated Contracting States:  
DE FR GB IT

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(30) Priority: 06.07.1984 US 628328  
06.07.1984 US 628335  
06.07.1984 US 628404  
06.07.1984 US 628409

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(43) Date of publication of application:  
29.05.1991 Bulletin 1991/22

(56) References cited:  
EP-A-0 052 288 FR-A-2 328 786  
US-A-4 233 360 US-A-4 294 241  
US-A-4 440 750

(62) Application number of the earlier application in  
accordance with Art. 76 EPC: 85304757.9

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US-A-4,066,083 from pigskin. The skin is finely divided, degreased using detergent, washed, and digested with pepsin to give a viscous suspension, and the collagen precipitated by addition of saturated salt solution. The precipitate is suspended in acid, reprecipitated as a fibrous white precipitate in salt solution, washed as many times as desired, and desalts by washing with alcohol. The purified collagen is suspended in acid solution and freeze dried. It is sterilized by  $\gamma$ -irradiation, which may degrade or cross link the preparation. This preparation is available commercially and has been used in a number of other bone repair studies.

Joos, U., et al, *Biomaterials* (1980) 1:23-26, utilized Collagenfleece® as an implant in artificially damaged rabbit mandibles and found that after 2 weeks, the defects were filled with cancellous bone particles and showed complete ossification after 4 weeks. Zetzmann, D., et al, *Schweiz Mschr Sabnheilk* (1982) 92:119 also achieved bone regeneration in facial surgery upon use of Collagenfleece® as an implant. Springorum, H. W., et al, *Z Orthop* (1977) 115:686 obtained similar results using Collagenfleece® in a cortical layer defect.

Jaffee, A., et al, *Archs Oral Biol* (1978) 23:415; ibid. (1982) 27:999 reported successful anchoring of acrylic tooth implants in dogs using collagen solutions which were prepared from dog skin by extraction with acetic acid and trichloroacetic acid/ethanol purification. The successful anchoring of the implants was intact after a year.

Cucin, R. L., et al, *New York State Journal of Medicine* (1979) 1856 used atelopeptide collagen from calf skin, which had been gamma irradiated, for rib repair in rabbits and, when supported by gelatin sponge material or with autologous bone dust, to repair skull holes in dogs.

A preparation of collagen, presumably still containing the telopeptides, and cross-linked by gamma irradiation was employed in filling tooth pulp cavities and as an under the skin "bone replacement" implant as disclosed in, respectively, EP-A-0012443 and EP-A-0012959. Another cross-linked preparation is seen in EP-A-0 052 288 (=US-A-4 597 762).

None of the foregoing collagen repair procedures are completely successful. Either inflammation occurs, particularly where xenogeneic collagen is used, or healing is unsatisfactory. The present invention provides an implantable collagen preparation which is capable of conducting the ingrowing bone repair tissue from dedicated bone cells into the defect whose repair is desired. Because xenogeneic collagen can be used, large amounts are obtainable and the method can be widely applied. In addition, the invention provides bone repair compositions which offer great versatility in being adaptable to a wide range of stress-bearing requirements.

The present invention provides a composition for repairing bone defects or reconstructing the skeletal matrix of a mammal, in particular a human, by implanting in the defect purified, non-immunogenic collagen which is derived, if desired, from a species other than

that being repaired. Thus, the invention provides for mediating the subject organism's natural mechanisms for bone defect repair by using a collagen preparation of general applicability which is highly purified, and which is successful in providing a matrix for new bone growth.

Fresh bone containing living osteoprogenitor cells is exposed to a bone defect and placed into contact with a preparation of collagen which is a composition derived from skin.

The skin-derived collagen is chiefly Type I collagen, includes a small amount of Type III and is typically obtained from calf skin. This type of collagen is commercially available under the trademark Zyderm® collagen soft tissue implant (ZCI) for use in removing wrinkles. The collagen preparation typified by ZCI is a reconstituted fibrillar form of atelopeptide collagen.

As is described for example in US-A-3949073, the skin-derived collagen is typically obtained from skin in a process wherein it has been dissociated into solubilized form, sterilized by filtration, and then reconstituted into fibrillar form after removal of the atelopeptides.

By varying the ratio of BCP to ZCI in a mixture of these peptides, the physical properties of the repair material can be adjusted to conform to the particular demands of the environment of the defect.

Thus in one aspect, the invention relates to a method of preparing a composition for repairing bone defects which method comprises preparing a suspension of purified reconstituted atelopeptide fibrillar skin collagen which is substantially free of cross linking, and lyophilizing the suspension. The collagen is preferably derived from calf skin.

Such a lyophilized preparation (lyophilized collagen gel or LCG) has favourable handling properties and can be extruded rolled or cast into sheets which are easily manipulated into implants. Alternatively it may be formed as a mat.

#### Modes of Carrying Out the Invention

##### A. Definitions

As used herein, "conductive" repair of bone defects refers to a process for replacing lost bone or for growing desired new bone, which involves the metabolism of previously committed osteoprogenitor cells. These cells are capable of producing cartilage and/or bone without induction by protein factors generally known as osteogenic or morphogenic. The process includes mechanisms whereby osteogenesis is directly effected by the committed cells, but not those wherein there is a requirement for added protein factors to produce committed cells.

"Consisting essentially of" means that the composition so defined derives all or substantially all of its relevant properties from the ingredient(s) as qualified.

It is realized that conversion of undifferentiated cells to osteoprogenitor cells may still be effected by indigenous proteins. However, as herein defined, "conductive"

capable of cross-linkage to lysine residues. Atelopeptide collagen must be cross-linked artificially, if it is so desired.

While all collagens share the foregoing characteristics, they have been subclassified into approximately ten types depending on the precise amino acid sequence in the individual chains of the triple helix, the carbohydrate content, and the presence or absence of disulfide cross-linking. The most common subtypes are Type I which is present in skin, tendon, and bone, and which is made by fibroblasts, and Type III which is found primarily in skin. Other types reside in specialized membranes or cartilage or at cell surfaces. Types I and III contain similar numbers of amino acids in their helices; however, Type III but not Type I contains two adjacent cysteines at the C-terminal ends of the triple helix which are capable of forming interchain cross-links.

Type I collagen contains one  $\alpha_2$  (I) and two  $\alpha_1$  (I) chains each of which contains 1014 amino acids in its triplet region; there are several carbohydrate moieties present on each chain. Type III collagen contains only  $\alpha_1$  (III) (3 chains) which contain 1026 residues in their triplet regions. As stated above, the presence in Type III of a pair of adjacent cysteine residues at the carboxy terminal end of the triplet region results in stability of the interchain cross-links. Both collagens contain short non-triplet ends (telopeptides). The reconstituted fibrillar atelopeptide collagen used in this invention contains the atelopeptide forms of both Type I and Type III; the bone collagen powder consists of the atelopeptide form of Type I exclusively.

#### The Skin-Derived Collagen

The atelopeptide reconstituted fibrillar skin collagen preparations useful in the compositions of this invention are typified by the purified calfskin-derived atelopeptide collagen reconstituted fibrillar suspensions sold commonly under the trademark ZYDERM® collagen implant (ZCI).

This and other preparations of skin-derived purified atelopeptide, reconstituted fibrillar collagen are well known in art. ZCI has been used extensively in soft tissue applications, including most prominently, the removal of wrinkles and depressed scars by injection of the preparation just under the skin. However, such preparations have not been used for bone repair except in conjunction with supporting materials which also contain OF and thus are directed to inductive bone repair.

As this collagen preparation is derived from calf skin, it contains mainly Type I collagen with approximately 1-5% Type III. The atelopeptide collagen is sterilized while still in solution by suitable filtration techniques and thus is not degraded or cross-linked. It is reconstituted into fibrillar form and packaged under sterile conditions.

#### The Lyophilized Skin Collagen

When this collagen material is lyophilized to form lyophilized collagen gel (LCG), it exhibits the ability to entrench itself in the implanted or filled cavity and to resist mobilization from the desired location due to its superior structural integrity. In addition, LCG matrices exhibit the desirable and necessary property of supporting the healing and regrowth of bone tissue into the implanted area. As described in further detail below, the LCG preparations of the invention may be prepared over a range of physical properties which are controlled by the pH, freezing rate, and concentration of the suspension during lyophilization. Whatever the properties desired, the LCG preparations of the invention are partially characterized in that they consist of atelopeptide reconstituted fibrillar collagen substantially free of impurities. If the preparation has been sterilized by microfiltration and processed under sterile conditions thereafter, it is also substantially free from cross-linking. The added lyophilization process results in a mat which is of sufficient cohesiveness to allow it to be easily cut simply using scissors or sharp blade, into the appropriate shape for clinical application. Wetting the mat produces a putty-like material which can be formed into any desired shape and placed into the defect.

We and others have disclosed forms of lyophilized collagen for medical applications. These collagen preparations, however, differ from those of the present invention as do their uses. Battista, US-A-3,471,598, discloses a lyophilized form of a preparation of an intermediate microcrystalline form of collagen, which is obtained by treatment of bovine skin with hydrochloric acid to swell and separate the collagen fibers. The collagen is not purified nor are the atelopeptides removed. The material so prepared is regarded as being suitable for water absorbent sponges; clearly, the impure nature and the immunogenicity of such preparations would make them relatively undesirable for direct medical use.

In addition, the acid used to prepare the intermediate form remains in the sponge. Kuntz, et al., US-A-3,368,911, disclose an alternate method of preparing absorbent collagen sponges so as to be devoid of the acid which, substitutes carbonic acid for the comparatively non-volatile acids used in Battista. While the preparation is disclosed to employ substantially pure collagen fibrils, the atelopeptides have not been removed. US-A-4,440,750 discloses a plastic dispersion of demineralized bone powder and reconstructed atelopeptide collagen. Miyata, US-A-4,294,241, discloses lyophilized collagen sheets for skin dressing. These sheets are prepared from atelopeptide collagen reconstituted into fibrils; however, they differ from the LCG of the present invention in that they are artificially cross-linked. Ries, in US-A-4,066,083, discloses the method of preparation of the commercially available product, Collagenfleece®, for wound treatment. Luck and Daniels, US-A-4,233,360, describe a lyophilized



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## RAPPORT DE RECHERCHE EUROPEENNE

Numéro de la demande

EP 88 40 2117

DOCUMENTS CONSIDERES COMME PERTINENTS			CLASSEMENT DE LA DEMANDE (Int. Cl.4)												
Catégorie	Citation du document avec indication, en cas de besoin, des parties pertinentes	Revendication concernée													
X, Y	EP-A-0 233 429 (P. FABRE MEDICAMENT) * Colonne 3, lignes 37-49; colonne 4, lignes 45-53; exemples; revendications * ---	1-10	A 61 K 47/00 A 61 K 31/415												
X	EP-A-0 138 216 (SUMITOMO CHEM. CO.) * Revendications 1,3,8 *	1-6,10													
Y	EP-A-0 224 453 (P. MORGANTI) * Colonne 1, lignes 19-28,35-44; revendications 1-3,5 *	1-10													
			DOMAINES TECHNIQUES RECHERCHES (Int. Cl.4)												
			A 61 K												
<p>Le présent rapport a été établi pour toutes les revendications</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Lieu de la recherche</td> <td style="width: 33%;">Date d'achèvement de la recherche</td> <td style="width: 33%;">Examinateur</td> </tr> <tr> <td>LA HAYE</td> <td>12-10-1988</td> <td>BERTE M.J.</td> </tr> <tr> <td colspan="2">CATEGORIE DES DOCUMENTS CITES</td> <td>T : théorie ou principe à la base de l'invention E : document de brevet antérieur, mais publié à la date de dépôt ou après cette date D : cité dans la demande L : cité pour d'autres raisons &amp; : membre de la même famille, document correspondant</td> </tr> <tr> <td colspan="2">X : particulièrement pertinent à lui seul Y : particulièrement pertinent en combinaison avec un autre document de la même catégorie A : arrière-plan technologique O : divulgation non-écrite P : document intercalaire</td> <td></td> </tr> </table>				Lieu de la recherche	Date d'achèvement de la recherche	Examinateur	LA HAYE	12-10-1988	BERTE M.J.	CATEGORIE DES DOCUMENTS CITES		T : théorie ou principe à la base de l'invention E : document de brevet antérieur, mais publié à la date de dépôt ou après cette date D : cité dans la demande L : cité pour d'autres raisons & : membre de la même famille, document correspondant	X : particulièrement pertinent à lui seul Y : particulièrement pertinent en combinaison avec un autre document de la même catégorie A : arrière-plan technologique O : divulgation non-écrite P : document intercalaire		
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removed. Bilateral 3 x 7 mm full thickness defects were placed in the parietal bones of the cranium with a dental burr. The lyophilized collagen as prepared in C.2.B was cut into strips slightly larger than the defects so as to effect a tight seal between the defect and the implant. Some of the strips were placed in the defects dry and allowed to hydrate in situ while others were prehydrated prior to implantation. After implantation, the scalp was repositioned and sutured. The rate of healing within the defects was evaluated by histology at 2 and 4 weeks post-implantation.

At 2 weeks, the implants were well infiltrated with connective tissue cells and blood vessels but new bone formation was very limited. While non-implanted defects of the type described in the previous paragraph did not heal and remained filled with soft tissue, the repaired defect showed areas of new bone forming throughout the implant by 28 days. Early evidence of fusion with pre-existing bone could be seen at the interface of the cut edges of the defect and the implant. By 56 days mature bone with well-defined marrow cavities were present throughout the defect.

LCG is easily cut and manipulated to form inserts capable of supporting conductive bone growth. The lyophilized reconstituted collagen fiber mats which are obtained provide a convenient and suitable material for this purpose.

#### Claims

1. A method of preparing a composition for effecting conductive repair of a bone defect in a mammal, which method comprises preparing a suspension of purified atelopeptide reconstituted fibrillar skin collagen which is substantially free of cross-linking, and lyophilizing the suspension.
2. The method according to claim 1 which includes forming the preparation into a sheet.
3. The method according to claim 1 or claim 2 wherein the suspension is prepared from calf skin.
4. A purified collagen preparation consisting essentially of a lyophilized reconstituted atelopeptide fibrillar skin collagen in the form of a sheet.
5. A purified collagen preparation according to claim 4 which is substantially free of cross-linking.
6. A process for preparing lyophilized collagen mats suitable for use as bone implants which process comprises:
  - (a) forming a suspension of purified atelopeptide reconstituted fibrillar skin collagen into sheets, and
  - (b) lyophilizing the resultant.

#### Patentansprüche

1. Verfahren zur Herstellung einer Zusammensetzung zur Durchführung einer konduktiven Reparatur eines Knochendefekts in einem Säugetier, welches Verfahren das Herstellen einer Suspension von gereinigtem, rekonstituiertem, fibrillärem Atelopeptidhautkollagen, das im wesentlichen frei von Vernetzungen ist, und das Lyophilisieren der Suspension umfaßt.
2. Verfahren nach Anspruch 1, das das Formen des Präparats zu einer Bahn umfaßt.
- 15 3. Verfahren nach Anspruch 1 oder 2, worin die Suspension aus Kalbshaut hergestellt wird.
4. Gereinigtes Kollagenpräparat, das im wesentlichen aus einem lyophilisierten, rekonstituierten, fibrillären Atelopeptidhautkollagen in Bahnform besteht.
- 20 5. Gereinigtes Kollagenpräparat nach Anspruch 4, das im wesentlichen frei von Vernetzungen ist.
- 25 6. Verfahren zum Herstellen lyophilisierter Kollagennäthen, die sich zur Verwendung als Knochenimplantate eignen, welches Verfahren die folgenden Schritte umfaßt:
  - (a) das Formen einer Suspension von gereinigtem, rekonstituiertem, fibrillärem Atelopeptidhautkollagen zu Bahnen und
  - (b) Lyophilisieren des Erhaltenen.

#### Revendications

1. Méthode de préparation d'une composition pour effectuer une réparation, par conduction, d'un défaut de l'os chez un mammifère, laquelle méthode consiste à préparer une suspension de collagène de peau fibrillaire reconstitué atélopeptidique purifié qui est sensiblement exempt de réticulations et à lyophiliser la suspension.
2. Méthode selon la revendication 1 qui consiste à former la préparation en une feuille.
3. Méthode selon la revendication 1 ou la revendication 2 où la suspension est préparée à partir de peau de veau.
4. Préparation de collagène purifié consistant essentiellement en un collagène de peau fibrillaire atélopeptidique reconstitué et lyophilisé sous la forme d'une feuille.
5. Préparation de collagène purifié selon la revendication 4 qui est sensiblement exempt de réticulations.